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(57) Abstract

The present invention relates to a method of distinguishing multiple alleles of a gene of the immunoglobin supergene family in a DNA sample. The present invention uses the single-stranded conformation polymorphism technique with unique conditions to distinguish and identify polymorphic alleles, such as DQα and DQβ alleles. The present invention is also useful for the identification of new alleles. Further, the method of the present invention can be used for typing tissues for transplantation.

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A METHOD FOR DISCRIMINATING AND IDENTIFYING ALLELES IN COMPLEX LOCI

BACKGROUND OF THE INVENTION

The ability to differentiate among alleles at polymorphic loci is a powerful means by which a specific allele may be shown to be associated with a particular phenotype. This has been shown to be useful in studying genetic associations with disease and in forensic analyses. The human major histocompatibility complex (MHC), contains sets of genes that encode products which are intimately involved in the initiation of immune responses. Among this set of genes are those designated HLA class II. The products of these genes function in presentation of antigens to T cells.

These genes and the HLA class I genes are amongst the most polymorphic known in vertebrates. In an attempt to understand the biological relevance of this heterogeneity, association between individual HLA gene products and resistance or susceptibility to over 40 diseases have been reported (reviewed in Bell et al. (1989) Adv. Human Genet. 18, 1-41).

Determination of the HLA alleles in various populations has been accomplished by serologic techniques, for example, using sera which recognize specific epitopes expressed on the surface of cells. Many alleles of the various genes have been sequenced revealing a considerable increase in polymorphism not recognized by serology. An efficient and reliable means for allele determination at the DNA level is necessary for not only the fully characterized alleles, but also for as yet unidentified alleles.

Several molecular techniques such as restriction fragment length polymorphism (Maeda et al. (1990) Human Immunol. 27, 111-121), allele-specific oligonucleotide (ASO) hybridization to amplified regions of the gene (Saiki et al. (1986) Nature (London) 324, 163-166), and more recently by an ELISA-based oligonucleotide ligation assay (Nickerson et al. (1990) Proc. Natl. Acad. Sci. USA 87, 8923-8927) have also been used for allele determination at various class II loci. These techniques, however, are inefficient, expensive and frequently erroneous. In addition, although some of these techniques are capable of detecting a single base difference in DNA sequence between two alleles, they are not likely to detect a new, undefined allele unless the variation happens to be at the specific site detected by the probe or the enzyme used for restriction. Furthermore, dependable HLA ASO typing requires very specific conditions including temperatures for hybridization and washing, salt concentrations of all solutions, and base composition of the probe used. In order to determine the genotype at one locus, generally several probes requiring various conditions are necessary.

Recently, a method was reported which can detect sequence variation, including single base changes as a shift in electrophoretic mobility (Orita et al. (1989) Proc. Natl. Acad. Sci. USA 86, 2766-2770). This technique is particularly rapid when the region of suspected variability is amplified by the polymerase chain reaction (PCR), then denatured and separated by electrophoresis to observe single-strand conformation polymorphism (SSCP; Orita et al. (1989) Genomics 5, 874-879). It

has been used and is well-suited for detection of mutant alleles which correlate with the presence of disorders such as cystic fibrosis (Dean et al. (1990) Cell 61, 863-870) and neurofibromatosis (Cawthon et al. (1990) Cell 62, 193-201). The present inventors have found that multiple alleles in complex genetic systems can be distinguished and new alleles identified using the SSCP technique. The method of the present invention is highly sensitive, distinguishes between alleles with single base changes and is faster than techniques involving hybridization with ASOs. In addition, the method of the present invention is particularly useful for identifying new alleles. The present invention can also be used for identifying HLA identical individuals more precisely than any technique now available.

Field of the Invention

The present invention relates, in general, to a method of distinguishing multiple alleles of a gene. In particular, the present invention relates to the identification of polymorphic alleles of the HLA class II genes.

SUMMARY OF THE INVENTION

It is an object of this invention to provide a method of identifying genetic variations which give rise to multiple protein forms expressed on cells.

It is another object of this invention to provide a process whereby many individuals can be typed simultaneously in relatively few steps, which process can be applied to any population and has the added advantage of identifying new alleles which

would be overlooked by the ASO hybridization typing method and by serology.

Further objects and advantages of the present invention will be clear from the description that follows.

In one embodiment, the present invention relates to a method of distinguishing multiple alleles of a gene of the immunoglobin supergene family. The DNA encoding the gene of interest in a sample is amplified and then denatured. The amplified DNA is then separated on a nondenaturing polyacrylamide gel consisting of 5% bis-acrylamide with 0-10% glycerol or Hydrolink gel matrix and the presence or absence of DNA bands is detected.

In another embodiment, the present invention relates to a method of distinguishing multiple alleles of a gene of the immunoglobin supergene family in a DNA sample by first grouping the alleles of the gene by oligonucleotide hybridization. The DNA encoding the gene is then amplified and denatured. The denatured DNA is separated on a nondenaturing polyacrylamide gel consisting of 5% bis-acrylamide with 0-10% glycerol or Hydrolink gel matrix and the presence or absence of DNA bands is detected.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Homoduplex patterns of DQA1 alleles. The second exon of the DQA1 locus was amplified from homozygous typing cell DNA representing 8 alleles. The double-stranded, amplified material was run on a 5% acrylamide gel at room temperature.

Figure 2. SSCP analysis of the DQA1 locus. The second exon of the DQA1 locus was amplified from homozygous typing cell DNA, and the products were subjected to SSCP analysis. Electrophoresis was performed using the following conditions: (A) 5% acrylamide at room temperature, (B) 5% acrylamide, 2% glycerol at 4°, (C) 5% acrylamide, 10% glycerol at 4°, (D) Hydrolink at room temperature.

Figure 3. SSCP analysis of the DQB1 locus. The second exon of the DQB1 locus was amplified from homozygous typing cell DNA representing 12 alleles using group-specific primers. The SSCP technique was performed on the amplified material. Electrophoresis was performed using the following primers and conditions: (A) DQB59, which primes DQB1*0601, *0602, and *0603, on 5% acrylamide at room temperature, (B) DQB59, on Hydrolink at 4°, (C) DQB60, which recognizes DQB1*0501, *0502, *0503, and *0604, on Hydrolink at 4°, (D) DQB60 on 5% acrylamide, 5% glycerol at 4°, (E) DQB72, which recognizes DQB1*0301, *0302, and *0303, on 5% acrylamide, 10% glycerol at 4°.

Figure 4. SSCP and sequence analysis of the DQB2 locus. (A) The second exon of the DQB2 locus was amplified from various DNA samples and the products were used in SSCP analysis. Electrophoresis was performed using a 5% acrylamide gel at 4°. (B) Sequencing was performed on two DNA samples determined to be homozygous at DQB2 based on SSCP analysis (9065 is represented on the upper sequence and A00 is the lower sequence). Dashes in the A00 sequence represent identity. Hha I restriction fragment sites for both alleles are shown.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of distinguishing multiple alleles of a gene. This method can also be used to identify new alleles. The method of the present invention distinguishes complex systems of polymorphic genes which differ at one or more positions. The present inventors have found that when the SSCP method is used with specific conditions for gel electrophoresis multiple alleles can be distinguished.

The SSCP method has been used for detection of mutant alleles which correlate with the presence of disorders such as cystic fibrosis and neurofibromatosis. As these genes are normally nonpolymorphic, the SSCP method can readily detect a new, mutant allele. The banding pattern in such cases is very simple. Unaffected individuals all have two bands with the same pattern while affected individuals will have up to four bands, two of which are generally identical to the normal allele pattern. However, for detection and identification of polymorphic genes, such as class II loci alleles (including DQA1 and DQB1), specific conditions are required due to the great polymorphism at each locus (for example, 8 for DQA1, and 12 for DQB1).

For example, with the DQA1 locus, all 8 alleles can be distinguished using two specific sets of conditions (Fig. 2B and 2D). Slight changes in gel composition (2% vs. 5% glycerol) made marked differences in banding patterns. Although many conditions were tested for separation of 12 DQB1 alleles, a more complex protocol is necessary for determination of these alleles. This protocol included group-specific oligonucleotide hybridization, amplification of specific groups with

discriminating primers, and separation of alleles by SSCP under various conditions. The present invention provides a protocol which is a sensitive means for identification of previously undefined alleles. Thus, the present method allows for the identification of genetic variations and for rapid typing of individuals, and tissue transplants.

In the method of the present invention, a portion of the gene of interest is amplified, by the polymerase chain reaction. Primers used in the amplification of the gene are selected so that the expected polymorphic region of the gene is amplified. Preferably, primers are selected so that the expected polymorphic region will not be at either end of the amplified segment (that is, at least 20 bases from the end of the expected polymorphic region). By positioning this region towards the middle of the segment, base differences more readily affect the structure of the DNA strand and thus are more readily distinguished by the method of the present invention. Examples of suitable primers for the DQα and the DQß alleles are given in Table 1.

After amplification, the DNA is denatured and the resulting single-stranded DNA is separated on a nondenaturing polyacrylamide gel. Gels consist of 5% bis-acrylamide-TBE with 0-10% glycerol added. Alternatively, a Hydrolink gel matrix can be used in place of acrylamide. Gels are run at about room temperature or at about 4°C. Various conditions used in running the gels dramatically alter the positions of bands and affect the ability of one to distinguish between multiple alleles. For example, differing DQα alleles can be identified by running a 5% acrylamide gel containing 2% glycerol at 4°C.

For loci having over 8 alleles, an additional initial step is required in order to identify the different alleles. Before amplification of such a gene, the alleles must be divided into subsets. This is most easily done using oligonucleotide hybridization [Saiki et al. (1986) Nature (London 324:163-166)]. Once the alleles have been grouped into subsets, the above described procedure is carried out on each subset. That is, the subsets are amplified with subset-specific primers (Table 1), the double-stranded DNA segments are then denatured and separated to distinguish the multiple alleles.

For example, twelve different DQβ alleles were analyzed individually by first dividing them into four groups (DQ1, -2, -3, and -4) by oligonucleotide hybridization. It was not necessary to divide DQB1*0201 and DQB1*04 beyond this step. Samples determined to be DQ1 or DQ3 were amplified a second time with group-specific primers (Table 1), and these were distinguished by SSCP using the following gel conditions: 1) Hydrolink at 4°C or 5% acrylamide at room temperature for DQB1*0601, *0602, and *0603, 2) both Hydrolink at 4°C and 5% acrylamide plus 5% glycerol at 4°C for DQB1*0501, *0502, *0503, and *0604, 3) 5% acrylamide containing 10% glycerol at 4°C for DQB1*0301, *0302, and *0303.

While the present invention is demonstrated with DQA1 and DQB1 loci, due to the structural and evolutionary similarities between members of the immunoglobin supergene family, the method can be used to distinguish multiple alleles of other members of the immunoglobin supergene family such as other MHC genes (HLA class I and HLA class II genes). By modifying the conditions

required for the HLA DQα and DQß locus, one skilled in the art can readily determine the gel electrophoresis conditions required to distinguish and identify multiple alleles of other genes in the immunoglobin supergene family. For example, the present invention is suitable for use in identifying alleles of the A, B, and C class I genes, other class II genes, such as the DPA1, DPB1, DRB1, DRB3, DRB4, T cell receptor genes, and immunoglobulin genes.

The method of the present invention was exemplified using the HLA locus. The present invention allowed the identification of the HLA DQα and DQß alleles. Eight DQα alleles and 12 DQß alleles were distinguished by amplifying the second exon of the genes in the presence of radioactive deoxynucleotides, denaturing the products with heat and separating the single strands by electrophoresis in nondenaturing gels. For DQα, it was possible to distinguish the 8 alleles with standard Bis-acrylamide or with a Hydrolink gel matrix (AT Biochem Malven, PA). Using the Hydrolink gel matrix, the molecules are separated by size (folding of the molecules however alters the run). In addition, using the present invention, a new allele at the DQB2 locus was discovered due to its unique banding pattern. Sequence analysis showed that this allele differed from that previously described by a single base pair in codon 25 of exon 2.

The present invention further relates to a method of typing individuals and donated tissue for transplant purposes. As is shown below for the DQα alleles, the present invention can be used to differentiate as many as 8 alleles of one gene and can therefore be used as a means of typing

individuals, for example, for HLA genes. The use of this technique for HLA typing individuals, particularly at DQA1, has the advantage of being very rapid and definitive relative to other available methods. For example, by hybridization techniques, a group of 50 individuals can be typed in about 5 days, whereas these individuals can be typed in 2 days by the present invention using half the amount of sample and fewer reagents.

Matching individuals at the HLA loci as closely as possible is essential for a successful transplantation. Using the method of the present invention, individuals can be typed and matched with available transplant tissues. Because the present technique is very sensitive and less prone to typing error than other typing techniques, it may be an appropriate replacement for the methods used in clinical laboratories for determination of transplantation matches.

There are several advantages to using the present invention for typing HLA genes over oligohybridization techniques presently used including specificity. Because several pairs of DQ alleles differ by only one base pair, oligotyping can be imprecise. It also appears that heterozygous individuals can sometimes type as homozygotes by oligotyping. For example, from a group of 20 individuals, two were oligotyped as homozygous A0101. Both of these individuals, however, were typed as A0101/A0401 by SSCP and this was confirmed by sequencing the amplified material. Subsequently, SSCP was performed on individuals who oligotyped as homozygotes in previous panels, and several errors based on SSCP were found. The present invention appears to be more sensitive in detecting both

alleles of a heterozygote which may have amplified differentially in that sample.

Another unique advantage to the use of the present invention for typing HLA genes is the discovery of previously unidentified alleles, as exemplified by the detection of a second DQB2 allele presented below. With the appropriate set of primers, determining the presence of HLA gene variants using SSCP would be very definitive and rapid relative to analysis of altered serologic reactivity and subsequent cloning and sequencing. The present invention can also be used to rapidly screen for MHC diversity in animal populations and for phylogenetic studies of related species.

Besides its usefulness in genotyping at HLA loci, the present invention has great potential for analyzing identity within HLA. The possibility of matching individuals for DP, DQ, DR, as well as class I genes using the present invention without actually having to type them may be a powerful and rapid tool for transplant analysis. Obtaining complex banding patterns using generic primers which may recognize multiple genes, such as DRß, would not be a hindrance in determining identity as it would be in genotyping. The number of amplifications necessary for analyzing identity would be limited, enhancing the efficiency of the assay.

The present invention is described in further detail in the following non-limiting examples.

EXAMPLES

The following protocols and experimental details are referenced in the examples that follow:

DNA Samples. DNA prepared from HLA D homozygous B lymphoblastoid cell lines (LCL) from the Xth International Histocompatibility Workshop (Yang et al. (1989) in Immunobiology of HLA., Vol. 1, ed. Dupont, B. (Springer-Verlag, New York) pp. 11-19) were used for reference. Additional DNA samples were prepared from LCL established from peripheral blood lymphocytes from a cohort of individuals in a multicenter study of HIV-1 infection.

DNA Amplification. Genomic DNA (500ng) was amplified as described previously (Saiki et al. (1988) Science 239, 487-491) for use in oligonucleotide typing in a volume of 50 ul using 4 units Taq polymerase (Digene Diagnostics, Silver Spring, MD), 0.2mM dNTP, and 180ng of each primer. Primers used for amplification and the alleles they recognized are listed in Table 1 below. Temperature cycling was carried out in a Programmable Thermal Controller (MJ Research, Inc., Watertown, MA) as follows: 30 cycles of 0.5 min 96°C, 1 min 57°C, 2 min 72°C for DQ or DXα and 30 cycles of 0.5 min 96°C, 1 min 63°C, 2 min 72°C for all DQ or DXβ primers.

Amplification for the SSCP assay was carried out as described above, except that 0.09 mM, as opposed to 0.2 mM dNTP was used in a total volume of 20 ul. One uCi [32P] CTP (Amersham Corp., Arlington Heights, IL) was added to the reaction mixture for labelling of the amplified product.

Oligonucleotide Typing. Following amplification, 5 ul of each sample were denatured in 0.15 M NaOH, 10 mM Tris-Cl, 1 mM EDTA and neutralized with an equal volume of 2 M NH_4-acetate and loaded onto nitrocellulose (Schleicher & Schuell, Keene, NH), using a Minifold II slot-blot apparatus (Schleicher & Schuell).

Blots were prehybridized in 50ml 5X SSPE (20X SSPE=3M NaCl, 0.2M Na H_2PO_4, 20mM EDTA), 5X Denhardt's solution and 0.5% Triton X-100 for 60 minutes and then hybridized for 30 minutes in 50ml of the same solution with the addition of the 200ng probe (probes and the alleles they recognize are listed in Table 2 below). Blots were washed for 10-20 minutes in 2X SSPE and 0.1% Triton X-100. Temperatures used for each probe are listed in Table 2. Blots were autoradiographed on Kodak X-Omat AR film (Eastman Kodak Co., Rochester, NY).

Single-Strand Conformation Polymorphism. Amplified DQα and DQβ DNA was digested with Eco RI and Alu 1, respectively, for 2 hr at 37°C. Samples were mixed with 2 volumes of 0.2% SDS, diluted 1:50 in stop solution (10 mM NaOH, 95% formamide, 0.05% bromphenol blue, 0.05% xylene cyanol, 0.3 ug/ml ethidium bromide), incubated at 97°C for 2 min, and placed on ice. Samples (2.5 ul) were electrophoresed on 20 cm 5% acrylamide-TBE with or without glycerol. Alternatively, a Hydrolink gel matrix (AT Biochem, Malvern, PA) was used in place of acrylamide. Gels were dried and placed on Kodak X-Omat AR X-ray film.

Direct DNA Sequencing. DQα and DQβ PCR products were digested with Eco Rl and Alu 1, respectively,

and electrophoresed on 20 cm acrylamide gels. The gels were stained with ethidium bromide, the bands were cut out, and eluted in 50 ul water at 65°C for 60 minutes. Single-stranded DNA was prepared by using five ul of eluate in a 40 cycle PCR with a 25- to 50-fold reduction in one of the primers. The DNA was purified on a Centricon 100 column (Amicon), precipitated, and dissolved in 14ul of water. Seven ul of this was then sequenced using Sequenase (U.S. Biochemicals). Sequences 5' to 3' and 3' to 5' were determined.

EXAMPLE 1

Determination of DQ Alpha Alleles.

Amplified DQα alleles from each DNA sample was first run on 5% acrylamide-TBE gels without denaturation in order to group the individuals with alleles A) A0101, A0102, A0103, B) A0301, C) A0201, A0401, A0501, A0601 or any combination of these three groups (Figure 1). Groups A and B are distinguishable from each other due to the higher G-C content of A0301, and groups A and B from C due to the presence of 3 more base pairs within the amplified region (exon 2) of these groups relative to group C. While this step was not necessary in genotype determination, it allowed verification of the SSCP findings.

Running denatured amplified DNA on a 5% acrylamide gel at room temperature did not distinguish all DQα alleles well enough to type heterozygous individuals (Fig. 2A), but when these samples were run at 4°C with the addition of 2% glycerol, all eight alleles could readily be distinguished in heterozygous individuals (Fig. 2B). Higher concentrations of glycerol (10%)

appeared to delineate the individual bands more precisely but did not differentiate all eight alleles (Figure 2C). The purpose of adding glycerol to the gel was simply to alter the position of bands in order to ascertain the best conditions for separation of alleles. Hydrolink was also capable of separating strands from the individual DQα alleles and these gels seemed to have the most definition relative to other conditions tried using polyacrylamide-based gels (Figure 2D). Heterozygotes for alleles amplified with the same set of primers showed a more complex banding pattern than those where only one allele was amplified (see Figure 2B for examples of SSCP with two alleles amplified by one set of primers).

EXAMPLE 2

Determination of DQ beta alleles.

The 13 DQβ alleles were divided into four groups (1)B0501, B0502, B0503, B0601, B0602, B0603, B0604 2)B0201 3)B0301, B0302, B0303 and 4)B0401, B0402) based on patterns of oligonucleotide hybridization of DNA, amplified with DQβ generic primers, with probes MC1, 2605, MC3, and 7007, respectively (see Table 2). Specific DQβ primers were then used to amplify DNA from the individuals in these groups and SSCP was used to distinguish the individual alleles. The 13 DQβ alleles could not be distinguished using a single set of conditions, so groups of beta alleles had to be analyzed individually. A protocol which worked well for typing DQβ involved oligohybridization to split individuals into the 4 major DQβ types (DQB1*01, 02, 03, and 04), amplification with the appropriate specific primers, followed by SSCP analysis.

The protocol was very definitive with the exception of separating products recognized by primer DQB60 (DQB1*0501, -0502, -0503, and 0604). DQB*0501 and -0502 could be distinguished using 5% acrylamide, whereas DQB1*0503 and -0604 were distinguishable under a variety of conditions including Hydrolink and 5% acrylamide with glycerol added, but not with standard 5% acrylamide alone. It is possible that altering the primer sequence slightly would allow the 4 alleles recognized by DQB60 to be separated using one set of conditions.

Figure 3A shows the patterns distinguishing alleles DQB1*0601, 0602, and 0603 from DNA amplified with the DQB59 primer and run on a 5% acrylamide gel in the absence of glycerol. These alleles could also be separated on Hydrolink (Fig. 3B). DQB60 was used to amplify the other alleles in group 1 (DQB1*0501, 0502, 0503, and 0604). Two different conditions were used to distinguish these four alleles. A Hydrolink gel and a 5% acrylamide gel containing 5% glycerol were used to distinguish 0503 from 0604 and 0501 from 0502, respectively (Figures 3C and 3D). Neither of these gels alone could distinguish all four alleles.

Primer DQB72 recognized alleles DQB1*0301, 0302, and 0303 and these were readily identifiable on an acrylamide gel containing 10% glycerol (Figure 3E). Amplification with the specific DQB primers listed in Table 1 always primed only the appropriate alleles as indicated by oligonucleotide hybridization and differential amplification using homozygous typing cell line DNA (Figure 3C).

EXAMPLE 3

Detection of new alleles.

One application of this technique is to identify new alleles. In analyzing this technique for typing various class II genes, it was found that the DQB2 gene, which has previously been thought to be nonpolymorphic (Bidwell, J. (1988) Immunol. Today 9, 18-23; and Bell et al. (1989) in Immunobiology of HLA, Vol II, ed. Dupont, B. (Springer-Verlag, New York) pp. 40-49) has at least two alleles. Using SSCP to analyze exon 2 of DQB2, two alleles were identified from a group of 8 homozygous typing cell DNA samples and 3 other DNA samples from individuals known to be heterozygous at DQß (Figure 4A).

DNA sequencing of samples shown to be homozygous for each allele confirmed the biallelic nature of the DQB2 locus (Figure 4B). The newly defined allele differed by one base pair in codon 25 of exon 2 (CGC --> CGG), both of which code for arginine. The DQB2 alleles did not appear to be in linkage disequilibrium with DQB1 because DNA from homozygous typing cells for DQB1*0602 and also for 0301 shown in Figure 4A are heterozygous for DQB2. Also, two different HTC DNA samples which both typed as DQB1*0502 did not share DQB2 alleles. A list of DQ haplotypes for the 11 individuals used in this analysis are shown in Table 3.

DNA amplified from the same stock DNA as those used for DQB2 analysis were amplified with DQA2-specific primers for exon 2 and run on SSCP. No differences were observed among these samples, suggesting a lack of polymorphism at this region of the gene.

Table 1. Oligonucleotide primers for DQ alpha and beta.

Primer	Allele	Sequence Pairs	SEQ ID NO.
DQB.5	All DBQ's	5'-CTCGGATCCGGGCATGTGCTACTTCACCA-3'	1
DQB.3		5'-GAGCTGCAGGTAGTTGTGTCTGCACAC-3'	2
DQB59	DQB*0601,0602,0603	5'-CCTCTGCAAGATCCCGCGGA-3'	3
DQB.5		5'-CTCGGATCCGGGCATGTGCTACTTCACCA-3	1
DQB60	DQB*0501,0502,0503,0604	5'-CCTCTGCAGGATCCCGCGGT-3'	4
DQB.5		5'-CTCGGATCCGGGCATGTGCTACTTCACCA-3'	1
DQB72	DBQ*0301,0302,0303	5'-ATAACCGAGAGGAGTACGCA-3'	5
DQB.3		5'-GAGCTGCAGGTAGTTGTGTCTGCACAC-3'	2
DQA.5	All DQA's	5'-GTGCTGCAGGTGTAAACTTGTACCAG-3'	6
DQA.3		5'-CACGGATCCGGTAGCAGCGGTAGAGTTG-3'	7
DQB.5	DXB	5'-CTCGGATCCGGGCATGTGCTACTTCACCA-3'	1
DXB.3		5'-GCAAGGTCGTGCGCAGCTCCG-3	8
DQA.5	DXA	5'-GTGCTGCAGGTGTAAACTTGTACCAG-3'	6
DXA.3		5'-CACGGATCCGCAGCGGTAGAGTTGGACT-3'	9

Table 2. Oligonucleotide probes for DQ alpha and beta.

Probe	Allele	Sequence	Annealing Temp	SEQ ID NO.
DQA1				
AG1	0101,0102,0103	5'-GCCTGGCGGTGGCCTGAG-3'	60c	10
DQA3410	0101	5'-GAGATGAGGAGTTCTACG-3'	58.5c	11
AG9-2	0102,0103,0501	5'-AGATGAGCAGTTCTACGTG-3'	60c	12
DQA2501	0101,0102,0401,0501	5'-TGGCCAGTACACCCATGA-3'	58.5c	13
AG2-3	0103	5'-ACCTGGAGAAGAAGGAGA-3'	52c	14
AG6	0201	5'-CTGTTCCACAGACTTAG-3'	53c	15
MCA3	0301	5'-TCTGGGCAGTACAGCCAT-3'	56c	16
DQA6903	0501	5'-ATCGCTGTCCTAAAACAT-3'	53c	17
AG8-1	0103,0201,0601	5'-TGGCCAGTTCACCCATGA-3'	58.5c	18
AG7-2	0401,0601	5'-GCTGTGACAAAACACAAATC-3'	56c	19
DQB1				
MC1	0501,0502,0503,0601 0602,0603,0604	5'-CCGCAGGGGCGGCCT-3'	54c	20
7001	0501,0502,0503	5'-GGCCCGGGCGTCGG-3'	54c	21
MC1.1	0502, 0501	5'-TGTACCGGGCAGTGACG-3'	54c	22
DQB5702	0502	5'-GCGGCCTAGCGCCGAGTA-3'	60c	23
7003	0601	5'-GACCCGAGCGGAGTTGG-3'	56c	24
5704	0602,0603	5'-GCGGCCTGATGCCGAG-3'	54c	25
2604	0603,0604	5'-CGTCTTGTAACCAGATACA-3'	50c	26
2605	0201	5'-GTCTTGTGAGCAGAAGCA-3'	52c	27
MC3	0301,0302,0303	5'-GAGAGGAGTACGCACGC-3'	56c	28
GH92	0301	5'-CGTGGAGGTGTACCGGGCG-3'	63c	29
5707	0302	5'-GCCGCCTGCCGCCGA-3'	56c	30
2603	0302,0303,0604	5'-CGTCTTGTGACCAGATAC-3'	54c	31
7007	0401,0402	5'-GAGGAGGACCGGGCGTC-3'	60c	32

Table 3. DQB1 and DQB2 haplotypes in 10 individuals.

Sample #	DQA1	DQB1	DQB2
9005	0101,0101	0501,0501	2,2
9008	0102,0102	0602,0602	1,2
9012	0102,0102	0502,0502	1,1
9027	0301,0301	0301,0301	1,2
9073	0301,0301	0303,0303	2,2
9103	0301,0301	0303,0303	2,2
9065	0103,0103	0603,0603	1,1
A48	0101,0501	0503,0201	1,2
A61	0301,0301	0301,0302	1,2
A83	0102,0401	0602,0402	2,2
A00	0102,0102	0502,0502	2,2

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Mann, Dean
Dean, Micheal
Carrington, Mary
White, Marga B.

(ii) TITLE OF INVENTION: A Method For Discriminating and Identifying Alleles in Complex Loci

(iii) NUMBER OF SEQUENCES: 35

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Cushman, Darby & Cushman
(B) STREET: 1615 L. Street, N.W.
(C) CITY: Washington
(D) STATE: D.C.
(E) COUNTRY: USA
(F) ZIP: 20036-5601

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Scott, Watson T.
(B) REGISTRATION NUMBER: 26,581
(C) REFERENCE/DOCKET NUMBER: WTS/5683/844 81/SLO

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (202)861-3000
(B) TELEFAX: (202)861-0944
(C) TELEX: 6714627 CUSH

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTCGGATCCG GGCATGTGCT ACTTCACCA 29

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAGCTGCAGG TAGTTGTGTC TGCACAC 27

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCTCTGCAAG ATCCCGCGGA

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCTCTGCAGG ATCCCGCGGT 20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATAACCGAGA GGAGTACGCA 20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTGCTGCAGG TGTAAACTTG TACCAG 26

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CACGGATCCG GTAGCAGCGG TAGAGTTG 28

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCAAGGTCGT GCGCAGCTCC G 21

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CACGGATCCG CAGCGGTAGA GTTGGACT 28

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCTGGCGGT GGCCTGAG 18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAGATGAGGA GTTCTACG 18

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGATGAGCAG TTCTACGTG 19

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGGCCAGTAC ACCCATGA 18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACCTGGAGAA GAAGGAGA 18

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTGTTCCACA GACTTAG 17

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TCTGGGCAGT ACAGCCAT 18

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATCGCTGTCC TAAAACAT 18

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TGGCCAGTTC ACCCATGA 18

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCTGTGACAA AACACAAATC 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCGCAGGGGC GGCCT 15

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGCCCGGGCG TCGG 14

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGTACCGGGC AGTGACG 17

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GCGGCCTAGC GCCGAGTA 18

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GACCCGAGCG GAGTTGG 17

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GCGGCCTGAT GCCGAG 16

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGTCTTGTAA CCAGATACA 19

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTCTTGTGAG CAGAAGCA 18

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAGAGGAGTA CGCACGC 17

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CGTGGAGGTG TACCGGGCG 19

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCCGCCTGCC GCCGA 15

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CGTCTTGTGA CCAGATAC 18

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGGAGGACC GGGCGTC 17

* * * * *

All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

WHAT IS CLAIMED IS:

1. A method of distinguishing multiple alleles of a gene of the immunoglobin supergene family in a DNA sample comprising the steps of:

i) amplifying said DNA encoding the gene with a primer specific for the polymorphic region of the gene;

ii) denaturing said amplified DNA;

iii) separating said DNA on a nondenaturing polyacrylamide gel consisting of about 5% bis-acrylamide with 0-10% glycerol or Hydrolink gel matrix; and

iv) detecting the presence or absence of DNA bands.

2. The method according to claim 1 wherein said DNA separation occurs at about room temperature or at about 4° C.

3. The method according to claim 1 wherein said gene is in the major histocompatibility complex.

4. The method according to claim 3 wherein said gene is a class II gene.

5. The method according to claim 4 wherein said class II gene is a DQα gene.

6. The method according to claim 5 wherein said DNA is amplified using at least one primer selected from the group consisting of:
5'-GTGCTGCAGGTGTAAACTTGTACCAG-3' (SEQ ID. NO:1);
5'-CACGGATCCGGTACGCAGCGGTAGAGTTG-3'(SEQ ID. NO:7);

5'-GCAAGGTCGTGCGCAGCTCCG-3'(SEQ ID. NO:8);
and
5'-CACGGATCCGCAGCGGTAGAGTTGGACT-3'(SEQ ID. NO:9).

7. The method according to claim 5 wherein said gene is a DQA1 gene.

8. The method according to claim 7 wherein said gene is a DQα gene and the DNA is separated on a 2% glycerol gel at 4°C.

9. A method of distinguishing multiple alleles of a gene of the immunoglobin supergene family in a DNA sample comprising the steps of:

i) grouping said alleles of said gene according to oligonucleotide hybridization specificity;

ii) amplifying said DNA encoding the gene with a primer specific for the polymorphic region of the gene;

iii) denaturing said amplified DNA;

iv) separating said DNA on a nondenaturing polyacrylamide gel consisting of 5% bis-acrylamide with 0-10% glycerol or Hydrolink gel matrix; and

v) detecting the presence or absence of DNA bands.

10. The method according to claim 9 wherein said gene is in the major histocompatibility complex.

11. The method according to claim 10 wherein said gene is a class II gene.

12. The method according to claim 11 wherein said class II gene is a DQß gene.

13. The method according to claim 9 wherein said DNA is amplified using at least one primer selected from the group consisting of:
5'-CTCGGATCCGGGCATGTGCTACTTCACCA-3'(SEQ ID. NO:1);
5'-GAGCTGCAGGTAGTTGTGTCTGCACAC-3'(SEQ ID. NO:2);
5'-CCTCTGCAAGATCCCGCGGA-3'(SEQ ID. NO:3);
5'-CCTCTGCAGGATCCCGCGGT-3'(SEQ ID. NO:4);
and
5'-ATAACCGAGAGGAGTACGCA-3'(SEQ ID. NO:5).

14. The method according to claim 12 wherein said gene is a DQB1 gene.

15. The method according to claim 12 wherein said gene is a DQß gene and the DNA is separated on a 5% acrylamide gel.

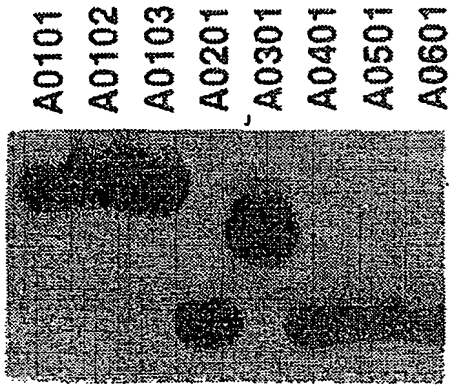

A0101
A0102
A0103
A0201
A0301
A0401
A0501
A0601
5% Acrylamide

FIG. 1

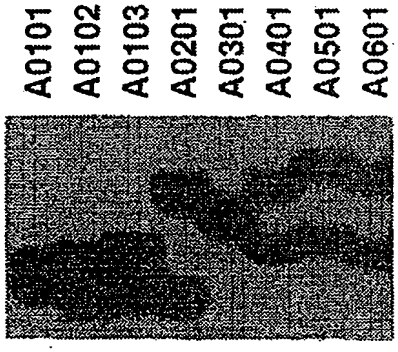

A0101
A0102
A0103
A0201
A0301
A0401
A0501
A0601
5% Acrylamide

FIG. 2A

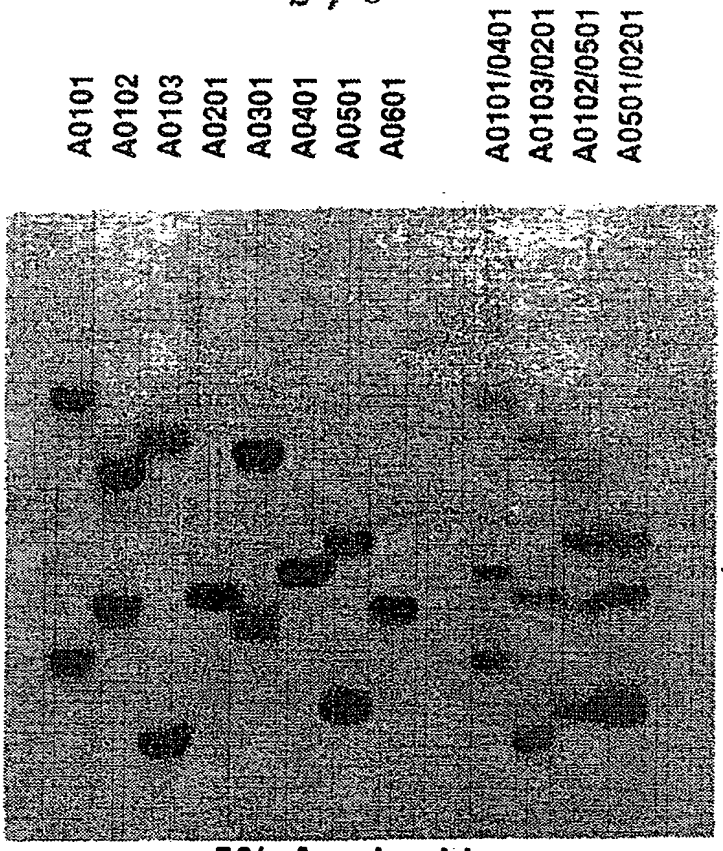


FIG. 2B

5% Acrylamide
2% Glycerol, 4C

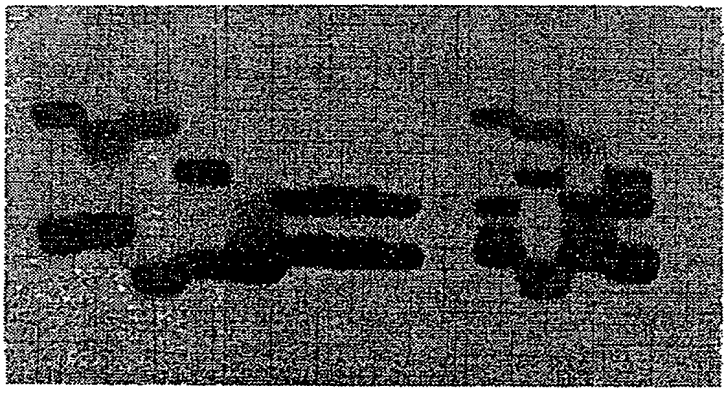
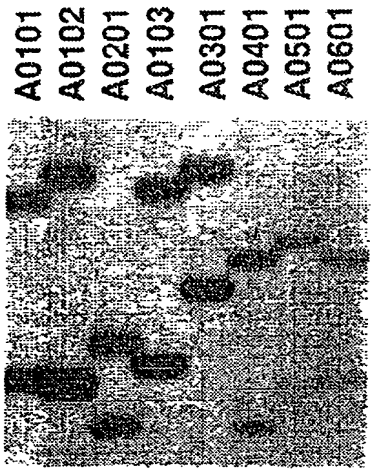


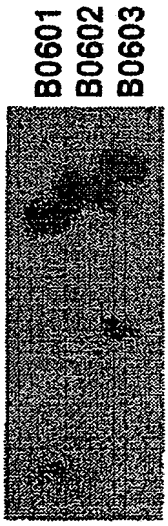
FIG. 2C

5% Acrylamide
10% Glycerol, 4C



Hydrolink
D5000

FIG. 2D

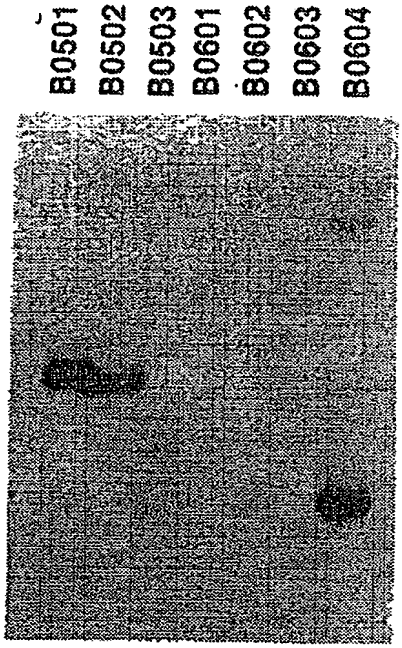


5% Acrylamide

FIG. 3A

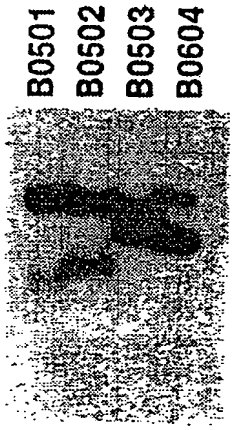
Hydrolink
4C

FIG. 3B



Hydrolink
4C

FIG. 3C



5% Acrylamide
5% Glycerol, 4C

FIG. 3D

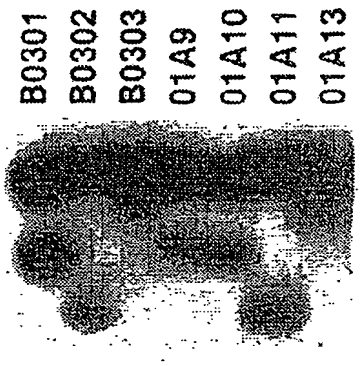


FIG. 3E

5% Acrylamide
10% Glycerol, 4C

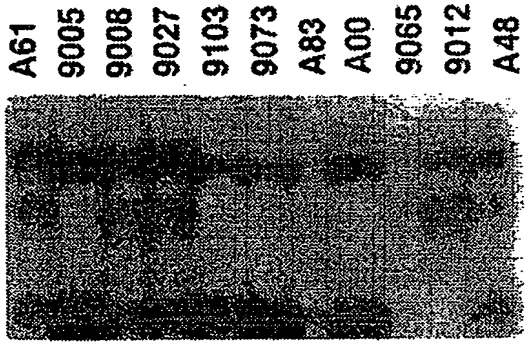
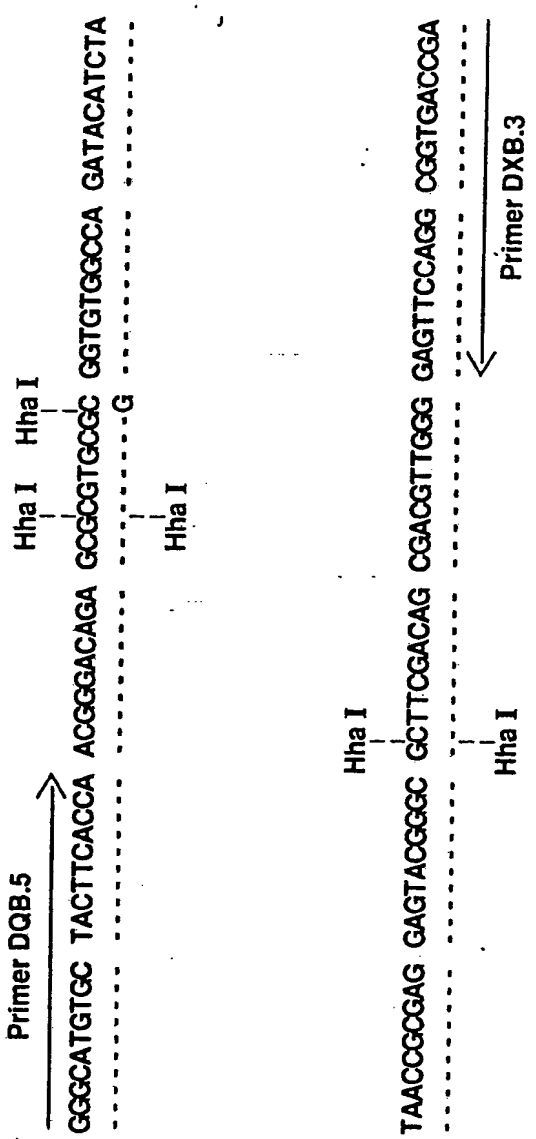


FIG. 4A

DQB2
5% Acrylamide
4C

FIG. 4B


Primer DQB.5
Hha I
Hha I
Hha I
Hha I
Hha I
Primer DXB.3
GGGCATGTGC TACTTCACCA ACGGGACAGA GCGCGTGCGC GGTGTGGCCA GATACATCTA
TAACCGCGAG GAGTACGGGC GCTTCGACAG CGACGTTGGG GAGTTCCAGG CGGTGACCGA

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/07153

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/68
US CL :435/6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, MEDLINE, UEMBL, GENBANK
search terms: hla, polymorphism, allele, dna

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF IMMUNOLOGY, Volume 139, No. 1, issued 01 July 1987, J. Schiffenbauer et al., "Complete Sequence of the HLA DQalpha and DQbeta cDNA from a DR5/DQw3 Cell Line", pages 228-233, see especially Figure 1-4.	1-15
Y	NATURE, Volume 324, issued 13 November 1986, R. K. Saiki et al., "Analysis of enzymatically amplified beta-globulin and HLA-DQalpha DNA with allele-specific oligonucleotide probes", pages 163-166, see entire document.	1-15
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 86, issued August 1989, S.J. Scharf et al., "Specific HLA-DQbeta and HLA-DQB1 alleles confer suseptibility to pemphigus vulgaris", pages 6215-6219, especially page 6216, column 1.	1-15
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 86, issued April 1989, M. Orita et al., "Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms", pages 2766-2770, see entire document.	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 SEPTEMBER 1992	Date of mailing of the international search report 07 OCT 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer LISA T. BENNETT Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*